Applicants' Response

Applicants' response to the office action of 12/05/2005 includes two sections: for each rejection: (1) a restatement of the Examiner's position which is found in 10 point italic font, and (2) applicant's reply in 12 point normal font.

Objection to the Specification

Claims 41 and 44 were objected to under 37 CFR 1.75(c), as being in improper dependent form for failing to further limit the subject matter of the previous claims, 1, 39 and 40. Applicants were required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. Claims 41 and 44 were drawn to a genetically engineered cell, while claims 1, 39, and 40 are drawn to a therapeutic delivery system comprising an electrical pulse generator operably linked to a genetically engineered cell. Since claims 41 and 44, drawn to a genetically engineered cell, do not recite the electrical pulse generator of claims 1, 39, and 40, claim 44 does not further limit claims 1, 39, and 40 .

Applicants have canceled Claims 41 and 42.

Claims 43 and 44 have been amended to be solely dependent on Claim 1. Claims 43 and 44 have also been amended to provide the proper antecedent basis to Claim 1 by including the preamble of the Claim 1. Applicants have added new claims 45-46 and 47-48 to be dependent on Claims 39 and 40, respectively, and to add the appropriate preamble of Claims 39 and 40.

Applicants believe the submitted amendments make these proper dependent claims and respectfully request the objection to Claims 41 and 44 be removed.

Claim Rejections under 35 USC § 112, - Enablement

*Claims 1-4, 7-26, and 39-43 were rejected under 35 U.S.C. 112, first paragraph, because the specification, while **being enabling for (i) electrically stimulated induction of gene expression in vitro using an electrical pulse generator operably coupled with cultured genetically engineered cells comprising a target gene operably coupled to an electrically responsive promoter and (ii) delivering to a subject an electrical pulse generator operably coupled to genetically engineered cells, wherein genetically engineered cells are transplanted into the subject** (Emphasis Added).*

While the subject claims described above were considered enabled, the Examiner did not currently believe the current claims reasonably provided enablement for (i) a therapeutic delivery system comprising an electrical pulse generator operably coupled with genetically engineered cells in a mammalian tissue, wherein said genetically engineered cells further comprise a target gene operably coupled to an electrically responsive promoter and (ii) a method of treating a patient comprising providing the patient with an electrical pulse generator operably coupled with genetically engineered cells in a patient tissue, wherein said genetically engineered cells further comprise a target gene operably coupled to an electrically responsive promoter. However, the Examiner has indicated the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims. While determining whether a specification is enabling, one considers whether the claimed invention provides sufficient guidance to make or use the claimed invention, if not, whether an artisan would require undue experimentation to make and use the claimed invention and whether working examples have been provided . Factors to be considered in determining whether a disclosure meets the enablement requirement of 35 USC § 112, first paragraph, have been described by the court in re Wands, 8 USPQ2d 1400 (CAFC 1988).

Wands states on page 1404, "Factors to be considered in determining whether a disclosure would require undue experimentation have been summarized by the board in Ex parte Forman. They include (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skills of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims."

The instant claims 1-4, 7-26 were found by the Examiner to be drawn to a therapeutic delivery system comprising electrically stimulated delivery of therapeutic products from cells that have been genetically engineered to express an electrically responsive promoter operably coupled to the genes encoding for the therapeutic products.

The Examiner found such language directed to a therapeutic delivery system is considered to directly embrace administering to animals a therapeutic agent in an amount sufficient such that the treatment of an animal having a condition associated with the therapeutic agent is achieved. Accordingly, the Examiner found the preamble language directed to "therapeutic delivery system" is considered to require support as outlined in 35 U.S.C. § 112 first paragraph such that therapeutic benefit is considered to be enabled for one seeking to make and use such a delivery system

While determining whether a specification is enabling, one considers whether the claimed invention provides sufficient guidance to make or use the claimed invention, if not, whether an artisan would require undue experimentation to make and use the claimed invention and whether working examples have been provided.

The Breadth of the Claims

The Examiner found the instant claims 1-4, and 7-26 were drawn to a therapeutic delivery system comprising an electrical pulse generator operably coupled with genetically engineered cells in a mammalian tissue, wherein said genetically engineered cells further comprise a target gene operably coupled to an electrically responsive promoter. The instant claims 39-43 are drawn to a method of treating a patient using the above-mentioned therapeutic delivery system. The aspects considered broad are: (i) the range of diseases to be treated, (ii) the range of target genes that can be used as therapeutic agents, and (iii) the range of genetically modified cells. The Examiner supports this conclusion by his analysis using the Wands factors, discussed below:

The Nature of the Invention

The nature of the invention was described by the Examiner to be the use of an electrical pulse generator to drive the expression of therapeutic agents to treat various diseases. Such invention has use in the art for treating various forms of disorders. However, the nature of such invention is within the broad genera of disorders treatment and disorders treatment does not generally enable Applicants' invention due to problems with the complexity and unpredictability of such disorders and also due to problems with using nucleic acid-based therapies, i.e., efficient and cell-specific delivery. The broad term disease encompasses distinct diseases that are caused by different genetic factors and result in different clinical manifestation. For example, the neurodegenerative disorders are caused by mutations of distinct genes that affect different areas of the brain, for example presenilin and amyloid precursor proteins (Alzheimer's disease), a-synuclein (Parkinson's disease), superoxide dismutase (amyotrophic lateral sclerosis or Lou Gehrig's disease), huntingtin (Huntington's disease) are the cause of distinct neurodegenerative diseases with distinct clinical manifestations (Hardy et al., Review, Science, 1998, 282:1075-1079). Also they could result in a similar symptom. How will therapeutic apply in these cases? The Artisan would not know what therapeutic agent to use to treat a symptom that can be caused by mutations in a number of different genes. With respect to this complexity and unpredictability of neurodegenerative diseases Hardy et al. teach:

> *"[T]he phenotype of a given mutation may not clearly predict a single expected clinicopathological entity. In the ataxias, abnormalities occur in any of a number of different genes, yet the clinical syndromes from the varied mutations are strikingly similar to each other.*
>
> *A key issue in the current research in neurodegenerative disease is that of select vulnerability.*
>
> *Only by considering this selectivity can we compare and contrast the biological mechanism of the disease. Each strikes a seemingly select group of neurons. Huntington's disease causes cell death in the caudate and results in chaotic movement. Parkinson's disease destroys cells in substantia nigra, resulting in rigidity and tremor and preventing initiation of movement. Amyotrophic lateral sclerosis damages the lower motor and pyramidal neurons and causes weakness and spacicity. Alzheimer's disease isolates the hippocampus and parietal lobes and prevents formation of new memory."*

Moreover, many diseases arising from injury or genetic abnormalities require one or more pharmacological agents for treatment. There are a growing number of polygenic diseases, such as hypertension, renal disease. How will therapeutic apply to polygenic diseases? The use of single drugs or single genes may often not work very well, due to the complexity of regulatory pathways. Tresco et al. (Advanced Drug Delivery Reviews, 2000, 42:3-27) teach:

> *"Although progress has been made in understanding the etiology and pathogenesis of diseases, in developing animal models and newer experimental therapeutics, few discoveries have been translated into clinically effective ways of delivering the multiple therapeutic agents obtained from living mammalian cells. Gene therapy, be it in vivo or ex vivo, so far has mostly been applied to monogenic diseases or in the lack of solid genetic evidence, diseases in which a single soluble compound is missing. Clearly, many more diseases are multifactorial in nature and consequently more difficult to treat. Furthermore, not all diseases are the result of missing soluble factors. The kinds of technical issues posed by more complicated diseases certainly have yet to be addressed. But gene therapy is a relatively new field that has only recently entered the clinical realm, and many fundamental issues still need to be addressed in the various disciplines that genetic engineering encompasses."*

Given these teachings, one skilled in the art would not know what gene to use to treat a clinical syndrome caused by mutations in a number of different genes, what genes to use to treat multifactorial diseases, or how to specifically deliver the therapeutic gene to the affected cells, without altering the non-affected cells. With respect to nucleic acid-based therapies, Applicants disclose that the therapeutic gene may be introduced into the target tissue as part of an expression vector in a

pharmaceutically acceptable carrier, either by direct administration to the tissue or by systemic delivery. Meyer et al. (Review, Cell. Mol. Biol. 2001, 47:1277- 1294) teach:

> *"Although gene therapy provides the hope and potential to revolutionize the future of medicine, this optimism must be tempered. Ongoing efforts to both quantitatively increase both gene transfer and expression to achieve improved therapeutic effect and to restrict the distribution and expression to relevant target tissues are under development. This includes enhancing the permeation of the vectors, development of targeted vectors that can be delivered systematically and regulating the level and target cell specificity of gene expression. Although these technologies are under development, advances in these areas will further improve the efficacy and safety of gene therapy vectors and further increase chances of success."*

Hence, from the nature of the invention, the Artisan would not reasonably predict that the delivery system claimed by the instant application could be used to treat diseases in general.

<u>The State of the Prior Art and the Level of Predictability in the Art</u>

The Examiner concluded that at the time the invention was made, and even in the present, the art of treating disorders was known to be unpredictable with respect to efficacy of delivering the nucleic acids to the targeted cells or tissues and a prolong expression of the therapeutic gene of interest.

The problems of nucleic acids based therapies are well known in the art, particularly with regard to the delivery systems, the inability to specifically deliver an effective concentration of a nucleic acid to a target cell, such that a target gene is expressed to a degree necessary to result in a therapeutic effect. For example, with respect to specific delivery and gene expression, Fisher A. (Review, Cell. Mol. Biol., 2001, 47:1269-1275) teaches:

> *"In spite of its logic, this therapeutic approach is complex considering the manner in which one must search in order to obtain a prolong expression of the therapeutic protein of interest. This objective contains several barriers: the difficulty in many cases of targeting cells (for example, the epithelial cells of the respiratory tract or of muscular fibers), the life duration of these cells, and the loss of expression of the transgene which is linked to several factors. For ten years, about 500 clinical gene therapy trials have been undertaken globally, with 80% of them occurring in the United States. Despite the fact that many of these experiments are more interested in issues of tolerance and pharmacology (phase I/II) that in efficacy, only very few have provided any proof of efficacy as of yet. Actually, this is easily explained by the difficulties of this therapeutic approach: it is necessary to obtain the expression of a potentially therapeutic gene that requires a good understanding of the disease's physiopathology in the targeted cells, at a level that is neither too low (efficacy) nor too high (toxicity). Ten years of clinical trials tests is not very much. It is true, however, that many actors in the research have largely underestimated the encountered difficulties."*

With respect to the problems encountered with the delivery systems, Gardlik et al. (Review, Med. Sci. Monit., 2005, 11: RA110-121) teach:

> *"The simplest way of gene delivery is injecting naked DNA encoding the therapeutic protein, but because of low efficiency there is a need to use special molecules and methods to improve gene delivery. Two kinds of vectors have been employed as vehicles for gene transfer. Viral vectors for gene transduction, such as retroviral, adenoviral, and adeno-associated viral vectors, and non-viral vectors for gene transfection, such as plasmids and liposomes. However, each vector has its own advantages and disadvantages: none of these types of vectors has been found to be ideal for both safe and efficient gene transfer and stable and sufficient gene expression"*

Therefore, at the time the instant invention was made, the therapeutic use of nucleic acids to treat disorders in general was a highly unpredictable art due to obstacles that continue to hinder the

therapeutic application of nucleic acids in vivo (whole organism) in general. Such obstacles include, for example, problems with delivery, target accessibility and stable and sufficient protein expression in the desired cells. For example, Lowenstein et al. (Review, Current Opinion in Pharmacology, 2004, 4: 91-97) teach:

> *"Could vectors, transgenes or transgenic proteins access the brain from the circulation? Delivery of viral vectors into the brain through a systemic route would be of great importance, but has so far neither been achieved nor explored in detail. Clearly this would be attractive because of the large areas of the brain that are affected in human neurodegenerative diseases."*

In his review article, Tuszynski (Expert Opin. Biol. Ther., 2003, 3:815-828) teaches:

> *"[T]here are also some relative potential disadvantages of in vivo gene therapy, including: Non-specificity of target cell infection – many different cell types can be infected when in vivo vectors are injected into CNS, including neurons, glia and vascular cells. Over expression of therapeutic genes such as nervous system growth factors in neurons could hypothetically exert deleterious effects by bypassing normal cell surface receptors binding mechanisms for growth factors. Thus, the potential of gene therapy to treat disease of the nervous system is vast and unprecedented, yet entirely hypothetical at this early stage of development." Given these teachings, the skilled artisan would not know a priori whether introduction of the expression vector in vivo by the broadly disclosed methodologies of the instant invention, would result in expression vector reaching the proper cell in a sufficient concentration and remaining for a sufficient time to provide successful therapy. One of skill in the art would not know how to deliver nucleic acids to an organism in such a way that would ensure an amount sufficient to stably and sufficiently express the therapeutic gene in the proper cell.*

Applicants also contemplate to administer therapeutic products by the transplantation of genetically engineered cells in conjunction with electro-stimulation. Tresco et al. teach:

> *"[T]ransplanted cells must be considered as a viable alternative to conventional delivery systems. Available information from studies conducted thus far have identified a number of critical questions that must be addressed in order for this promising approach to make transition from a boutique laboratory to a common component of the medical armamentarium".*

Some of the questions to be addressed are : should the therapeutic agent be produced constitutively or should it be released in a regulated manner; what type of cell is optimal as a delivery vehicle; is the therapeutic effect to be achieved over short-time or over a more prolonged interval; should the transplanted cells be allowed to integrate into host tissue; or are they to be maintained physically isolated; do transplanted cells have the potential to migrate away from the site of transplantation?

With respect to the above questions, Tresco et al. teach that (i) many diseases, such as enzymatic deficiency disorders, do not require regulated delivery; in contrast, in certain situations, such as diabetes, regulated delivery is critical (p. 4, column 2, fourth paragraph); (ii) choosing the optimal type of cell is very important, depending on the desired outcome: cell phenotype, or biosynthetic activity of the cell or the turnover rate in vivo each has to be considered for optimal results (p. 5, column 1, second paragraph), (iii) the inherent turnover of the cell type delivered is the key difference between short-term or long-term treatments; for genetically modified cells the efficacy of the original genetic modification is critical . The inactivation of the transgenes is an important obstacle for the treatment of chronic disorders by gene therapy (p. 5, column 1, third paragraph), (iv) if it is not possible to transplant autologous cells, cell encapsulation might be required to protect the donor cells from destruction by the immune system (p. 5, column 1, last paragraph), and (v) the migratory capacity of the cell used as a delivery vehicle must be considered; for systemic delivery of agents one desires to have cells migrate, for local delivery one desires to have cells remain at a specific site (p. 5, column 2, second paragraph).

Given these teachings, the skilled artisan would not know a priori whether introduction of any genetically modified cell in vivo by the broadly disclosed methodologies of the instant invention, would

result in therapeutic agent reaching the proper target in a sufficient concentration and remaining for a sufficient time to provide successful therapy. One of skill in the art would not know what type of genetically modified cell to deliver to an organism in such a way that would ensure an amount sufficient to stably and sufficiently express the therapeutic gene at the proper site, to treat diseases in general.

While the intent is not to say that nucleic acids or genetically modified cell transplants can never be used to treat diseases, the intent is to provide art taught reasoning as to why the instant claims are not enabled. Given this unpredictability, particularly when taken with the lack of guidance in the specification, it would have required undue experimentation to practice the claimed methods in vivo in an enormous number of organisms as broadly or generically claimed, with a resultant treatment of diseases in general, as claimed.

In fact, the state of the art is such that successful delivery of nucleic acids in vivo or in vitro, such that they provide the requisite biological effect to the target cells/tissues/organs, must be determined empirically. Methods of gene therapy using nucleic acids in general in vivo are unpredictable with respect to delivery of the nucleic acid molecule such that the nucleic acid molecule is targeted to the appropriate cell/organ, at a bioeffective concentration and for a period of time such that the nucleic acid molecule is effective in, as in the instant application, treatment of diseases in general.

Similarly, the state of the art for successful delivery of therapeutic agents via genetically modified cell implants to provide the requisite biological effect to the target cells/tissues/organs, must be determined empirically. Methods of gene therapy using genetically modified cell implants are unpredictable with respect to delivery of the therapeutic molecule such that the therapeutic molecule is targeted to the appropriate cell/organ, at a bioeffective concentration and for a period of time such that the therapeutic molecule is effective in, as in the instant application, treatment of diseases in general.

<u>The Amount of Direction or Guidance/The Existence of Working Examples</u>

The Examiner concluded the specification does not provide the guidance or the working examples required to overcome the art-recognized unpredictability of using nucleic acids or genetically modified cell implants in therapeutic applications in any organism. The field of nucleic acids/genetically modified cell implants therapeutics does not provide that guidance, such that the skilled artisan would be able to practice the claimed therapeutic methods.

It is noted that specification discloses one in vitro example for using electrical pulse to stimulate the expression of the luciferase gene in cultures of genetically engineered cells comprising the luciferase gene under the control of the ANF promoter. It is also noted that the specification discloses one example of electrical stimulation of dogs that received a transplant of cultured skeletal myoblasts after experimentally induced myocardial infarction. However, the myoblasts used for transplantation were not genetically modified, i.e., did not comprise a construct composed of a target gene operably linked to an electrically-responsive promoter, therefore the limitation recited in the instant claims is not met.

Given the diverse and unpredictable outcome of using the disclosed delivery system to treat diseases, the specification does not appear to provide sufficient guidance and/or working examples that specifically address the use of this delivery system as being effective in treating various diseases in animals to enable one of ordinary skill in the art to use such delivery system without undue experimentation. Gilmour et al. (Developmental Biology, 1995, 168:416-428) teach:

> *"Data presented here indicate that the enhancer activity of this region of the y subunit gene seen in Sol8 muscle cells and MyoD-cotransfected 1OT1/2 fibroblasts does not exist in rat primary muscle cultures indicating physiological differences between the established Sol8 cell line and primary muscle culture. The data presented here indicate that, although analysis of transcriptional activity in immortalized cell lines is a valuable tool due to the homogeneity of the cultures and ease of preparation, further analysis in primary cell cultures (or animal models) is necessary in order to draw more valid conclusions as to the relevance of experimental results to transcriptional mechanisms in vivo".*

Conclusion
In conclusion, the presently claimed invention only provides enough of a disclosure to allow for an Artisan to : (i) electrically stimulate induction of gene expression in vitro using an electrical pulse generator operably coupled with cultured genetically engineered cells comprising a target gene operably coupled to an electrically responsive promoter (claims 1-4 and 7-26), and (ii) delivering to a subject an electrical pulse generator operably coupled to genetically engineered cells, wherein genetically engineered cells are transplanted into the subject (claims 39-43).

Applicants have submitted a series of amendments to independent Claims 1 39, and 40. The series of amendments include first, the introduction that the genetically engineered cells comprise the target gene operably coupled *in vitro* to an electrically responsive promoter." Second, it has been introduced into the claims that the electrical pulse generator is implanted, which in turn is coupled to said genetically engineered cells. Third, the claims have been amended to indicate that the cells are implanted in a mammalian tissue. Fourth, Claims 1, 39, and 40 have been amended to include that the system is capable of enhancing transcription. Additionally, Applicants have entered amendments to the Claims to indicate the target gene that has been operably coupled in vitro to a heterologous electrically responsive promoter. (emphasis added to highlight the submitted amendments)

The Examiner has stated that the application was enabled under these conditions; however, Applicants' reserve the right to introduce additional claims at some future time, but make these current amendments to forward prosecution. Based on the current set of amendments, Applicants request that the present rejection under 112, be removed.

Claim Rejections - 35 USC § 102, over Gilmour et al.

Claims 1-4, 11, 23, 24, and 44 were rejected by the Examiner under 35 U.S.C. 102(b) as being anticipated by Gilmour et al . (Developmental Biology, 1995, 168:416-428). Gilmour et al. was cited for teaching electrical pulse stimulation of primary muscle cells transfected with luciferase operably coupled to the nicotinic acetylcholine receptor y subunit promoter (p. 418, column 2, Transfection and Electrical Stimulation). Gilmour et al. teach that the nicotinic acetylcholine receptor y subunit promoter mediates suppression of transcription in response to electrical activity (p. 421, column 1, last paragraph, column 2, first paragraph, p. 423, Fig. 4, and p. 424, column 2, Discussion). The pulses do not appear to damage the cells and thus, are considered to be subthreshold-applied pulses.

Since the art teaches electrically stimulation of gene expression in vitro using an electrical pulse generator operably coupled with cultured genetically engineered cells comprising a target gene operably coupled to an electrically responsive promoter, the claimed inventions are anticipated by the above-cited art.

In order to be an anticipatory reference, the reference must teach each element of the claimed invention. As the Examiner has pointed out "Gilmour et al. teach that the nicotinic acetylcholine receptor *y* subunit promoter mediates **suppression** of transcription in response to electrical activity." (emphasis added). Applicants have amended Independent Claims 1, 39 and 40 to make clear that the claim invention is directed to enhancing transcription.

Further Applicants wish to further point out that the claimed invention is directed to an implanted pulse generator that couples to the genetically engineered cells. These features are also neither taught nor suggested by Gilmour for the delivered therapy. No where does Gilmour teach or suggest the use of an implanted pulse generator or that the pulse generator is coupled to the implanted genetically engineered cells.

Based on the submitted amendments and arguments, Applicants respectfully request removal of the instant novelty rejection over Gilmour et al.

Claim Rejections - 35 USC § 102, over Yanagida et al.

Claims 1-4, II, 13, 23, 24, and 44 are also rejected by the Examiner under 35 U .S.C. 102(a) as being anticipated by Yanagida et al. (Journal of Biotechnology, April 14, 2000, 79 : 53-61). Yanagida et al. teach 3T3 cells transfected with luciferase operably coupled to the hsp70 promoter (p. 56, column 2, last paragraph) Yanagida et al .teach that electrical pulse stimulation of the hsp70 promoter results in induction of luciferase expression (p. 55, column 2 and p. 58, Fig. 5). The pulses do not appear to damage the cells and thus, are considered to be subthreshold-applied pulses

Since the art teaches electrically stimulation of gene expression in vitro using an electrical pulse generator operably coupled with cultured genetically engineered cells comprising a target gene operably coupled hsp70 promoter, the claimed inventions are anticipated by the above-cited art.

Applicants distinguish the present invention (as amended) over Yanagida. Applicants claimed invention is directed in part to "A therapeutic delivery system comprising an implanted electrical pulse generator operably coupled with implanted genetically engineered cells in a mammalian tissue." No where does Yanagida teach use an implanted pulse generator, or that the implanted pulse generator is coupled to cells that have been implanted in a mammalian tissue.

Because Yanagida fails to teach all the claimed elements of the invention, Yanagida fails to be a novelty destroying reference. Applicants, thereby, respectfully request removal of the present rejection of novelty over Yanagida.

Claim Rejections - 35 USC § 103; Lee et al. in view of Kanno et al.

Claims 1-4, 7- 11, 13, 14, 23-25, and 39-44 were rejected by the Examiner under 35 U .S.C. 103(a) as being unpatentable over Lee et al. (Circulation, Aug, 22, 2000, 102 : 898-901), in view of Kanno et al . (Circulation, 1999, 99: 1682-1687).

Lee et al. teach implantation of primary murine myoblasts expressing the murine VEGF gene from a retroviral promoter into the ventricular wall of immunodeficient mice (Abstract, p. 899, column 1, Materials and Methods). Lee et al. does not teach an electrically responsive promoter or induction of VEGF by electrical stimulation. Kanno et al. teach induction of VEGF in electrical pulse stimulated murine myoblast cell line C2C12 (p. 1682, column 2, Methods, and p .2684, column 2, second and third paragraphs). Kanno et al. do not teach implantation of myoblasts. Kanno et al. that their procedure could be used in therapeutic angiogenesis, for example in ischemic diseases (Abstract, p. 2686, column 2, last paragraph). It would have been obvious to one of skill in the art, at the time the invention was made, to use the method of Lee et al. to transplant genetically engineered myoblasts expressing VEGF and increase VEGF expression by generating an electrical pulse, as taught by Kanno et al ., with a reasonable expectation of success . The motivation to do so is provided by Kanno et al. who teach gene therapy as relevant for therapeutic angiogenesis and the importance of transplanting VEGF-expressing cells in the ischemic area only since localized, controlled expression of VEGF induced by electrical pulse stimulation, thereby inducing the activity of the promoter which would activate local VEGF production, salvaging the ischemic area (Abstract, and p. 2686, column 2, last paragraph) and by Lee et al. who teach potential toxicity of unregulated myoblasts-mediated VEGF expression (p. 900) .

Thus, the Examiner concludes that the claimed invention was prima facie obvious at the time the invention was made.

Applicants respectively traverse that Lee et al. in view of Kanno et al. 1) teach the claimed invention or 2) that there is sufficient motivation to combine these references.

As the Examiner points out, Lee teaches delivery of a VEGF gene through a retroviral promoter into the ventricular wall of immunodeficient mice. No where does Lee teach that the retroviral promoter is an electrically responsive promoter. In order to overcome the deficiencies of Lee, the Examiner has cited the reference by Kanno et al. Kanno et al. was cited for teaching the induction of the VEGF gene in electrical pulse stimulated C2C12 cells as an electrically responsive promoter system.

Applicants wish to point out that Kanno et al. only teaches induction of expression of the endogenous VEGF gene using electrical stimulation in non-genetically engineered cells. It was not shown in Kanno et al. to be effective in engineered cells, nor was it shown to be applicable in heterologous promoter systems. Both these limitations are contained in the claims.

In addition, after reading Kanno et al. there would be no motivation for transfecting the VEGF gene into the tissue to enhance expression of this gene by electrical stimulation, wherein the gene is already present in the tissue and responsive to electrical stimulation. Because the gene is present and shown to be responsive to electrical stimulation there is no motivation to combine these systems. Electrical stimulation of the endogenous gene would have achieved the desired without creating the required genetically engineered cells and then coupling these to an implantable pulse generator. Finally, neither Lee et al. or Kanno teach heterologous promoter constructs using an electrically responsive promoter element.

Because Lee et al. in view of Kanno fails to teach or suggest the claimed invention or that there is sufficient motivation to combine these references, Applicants' respectfully request that the instant rejection be removed.

Claim Rejections - 35 USC § 103; Gilmour et al. or Yanagida et al. in view of US20010031919A1.

Claims 1, 7-10 were also rejected under 35 USC 103(a) as being unpatentable over either Gilmour et al . or Yanagida et al ., as applied to Claim 1, and further in view US20010031919 Al.

Neither Gilmour et al. or Yanagida et al ., teach explicitly the limitations of using a pacemaker or any electrical device implanted or externally controlled so as to provide an electrical pulse for gene expression in desired cells.

However, at the time the invention was made, the US20010031919 A1 reference did teach that a pacemaker can be attached to a gene delivery tool (Claim 22, par . 0136). Thus, the Examiner concludes it would have been obvious for one of ordinary skill in the art to employ any know device for providing a desired amount of pulses as taught by the primary reference, e.g. ., Gilmour et al. or Yanagida et al.

One of ordinary skill in the art would have been motivated to employ a pulse making device such as a pace maker implanted internally or externally because such devices are well known in the art and the use of the device would provide the sources of pulses as required for providing a stimulation of gene expression, which electrical pulse stimulation of a promoter is crucial for modulation of gene expression as taught by the primary reference . One of ordinary skill in the art would have a reasonable expectation of success in making and use such as the combined composition because medical devices such as pace makers are proven to provide any amount of pulses as desired in stimulating gene expression. Thus, the claimed invention was prima facie obvious at the time the invention was made.

As previously indicated, no where does Yanagida teach the use of an implanted pulse generator, or that the implanted pulse generator is coupled to transplanted cells in a mammalian tissue. Similarly, no where does Gilmour teach an implanted pulse generator that coupled to genetically engineered cells. This feature is also neither taught nor suggested by Gilmour or Yanagida in view of US20010031919A1 for the delivered therapy.

US20010031919 (the '919 publication) does not cure this problem. The '919 publication only teaches a medical imaging system that can be used with either a gene delivery tool or used with a pacemaker. There is no statement or teaching for use of a pulse generator with a gene delivery tool, particularly wherein the cells are responsive to the pulse generator or that the cells contain an electrically responsive element coupled to a heterologous gene. Further, as previously indicated Gilmour et al. teach that the nicotinic acetylcholine receptor *y* subunit promoter mediates **suppression** of transcription in response to electrical activity." (Emphasis added)

Because Gilmour or Yanagida et al. in view of the '919 publication does not collectively teach or suggest the claimed invention, Applicants respectfully request the present obviousness rejection in view of these references be removed.

Claim Rejections - 35 USC § 103; Gilmour et al. in view of Allen.

Claims 1, 12, 14, and 16-22 are rejected under 35 USC 103(a) as being unpatentable over Gilmour et al., as applied to claim 1, in view of Allen (Ann Thorac Surg. 1999, 68:1924-1925).

The Examiner points out that Gilmour et al. does not teach the limitation of using a tissue specific promoter. However, at the time the invention was made, Allen did teach organ-selective local delivery of therapeutic genes (Abstract, page 1924 bridging page 1925, column 1, first paragraph). Thus, it would have been obvious for one of ordinary skill in the art, at the time the invention was made, to genetically alter cells with a construct comprising the electrically responsive enhancer of Gilmour et al. and link it to a tissue specific promoter for local delivery, as taught by Allen, with a reasonable expectation of success.

One of ordinary skill in the art would have been motivated to employ such a chimeric enhancer-promoter construct in conjunction with an electrical pulse generator for controlled delivery of genes to specific organs/tissue, since the electrical pulse stimulation of a promoter is crucial for modulation of gene expression as taught by the primary reference. One of ordinary skill in the art would have a reasonable expectation of success in making and use such as the combined composition because electrically responsive enhancers are proven to promote gene expression as desired, upon electrical stimulation.

Thus, the claimed invention was prima facie obvious at the time the invention was made.

As previously discussed in our arguments regarding Gilmour as a § 102 reference, no where does Gilmour teach an implanted pulse generator that couples to the claimed genetically engineered cells. Particularly, Gilmour does not teach a heterologous electrically responsive promoter element attached to a gene of interest in transplanted cells. It is incorrect that Gilmour teaches an electrically responsive enhancer. Gilmour actually taught suppression after electrical stimulation. Adding the tissue specific promoter element taught by Allen fails to either cure the defects of Allen or achieve the claimed results of the claims.

Thereby, Applicants respectfully request the present rejection of Gilmour et al. in view of Allen be removed.

Claim Rejections - 35 USC § 103; Gilmour et al. in view of McDonough et al.

Claims 1 and 15 were also rejected under 35 USC 103(a) as being unpatentable over Gilmour et al., as applied to claim 1, in view of McDonough et al. (J. Biol. Chem. 1997, 272:24046-24053).

The Examiner points out that Gilmour et al. does not teach the limitation of using an electrically enhancer element selected from the ANF 5' non-coding region. However, at the time the invention was made, the Examiner points out that McDonough et al. taught elements derived from the ANF 5' non-coding region driving the expression of the luciferase gene upon electrical stimulation (page 24047, Experimental Procedures). Thus, the Examiner concludes it would have been obvious for one of ordinary skill in the art, at the time the invention was made, to replace the enhancer of Gilmour et al. with the enhancer of McDonough et al., with a reasonable expectation of success. One of ordinary skill in the art would have been motivated to employ such a chimerical enhancer-promoter containing in conjunction with an electrical pulse generator for controlled delivery of genes, and would have been expected to have a reasonable expectation of success in making and use such as the combined composition because the enhancer element selected from the ANF 5' non-coding region is proven to promote gene expression as desired, upon electrical stimulation.

Thus, the claimed invention was prima facie obvious at the time the invention was made.

Applicants wish to point out that the standing claims are distinguishable and patentable over Gilmour in view of McDonough. No where does Gilmour teach an implanted pulse generator that couples to genetically engineered cells. Although McDonough teaches the use of electrical stimulation, it fails to teach or suggest a device that could be used as an implanted pacemaker. In the cited McDonough reference, is a cite to his earlier Journal of Biochemistry Paper for the device used to stimulate cells (see previously submitted IDS for McDonough et al., J. Biol. Chem. 269(13):9466-9472, 1994). This device shows cultured cells utilizing silver:silver chloride electrodes inserted through holes in the lid of a multiculture dish. The circuits were completed by linking the individual wells with thin strips of 1% agarose gel permeated with culture medium. Although McDonough refers to pacing cells, it clearly is in context of pacing cultured cells. No where in McDonough or Gilmour is there a clear teaching or suggestion of use of an implantable electrical stimulation device or the requirements for such a device. Further, neither Gilmour nor McDonough teach the use transplanting these cells in a therapy, nor coupling the implanted cells to a pulse generator.

Applicants respectfully request after review of the teaching of Gilmour in view of McDonough that the present rejection be removed.

Claim Rejections - 35 USC § 103; Gilmour et al. in view of Kaye et al.

Claims 1 and 26 are rejected under 35 USC 103(a) as being unpatentable over Gilmour et al., as applied to claim 1, in view of Kaye et al. (Circ. Res., 78:217- 224, 1996).

Gilmour et al. do not teach a coding sequence selected from the group recited in claim 26. However, at the time the invention was made, Kaye et al did teach activation of constitutive nitric oxide synthase (NOS) in rat myocytes upon electrical stimulation (Abstract, page 219, bridging page220, column 1).

Thus, it would have been obvious for one of ordinary skill in the art, at the time the invention was made, to employ a genetically engineer a cell with a construct comprising the nicotinic acetylcholine receptor y subunit promoter of Gilmour et al .linked to a cDNA encoding for NOS to modulate NOS expression upon electrical stimulation, with a reasonable expectation of success. One of ordinary skill in the art would have been motivated to employ such a chimeric construct in conjunction with an electrical pulse generator for controlled expression of NOS, since Kaye et al. teach that NOS participates in the regulation of contractile function of cardiac muscle via nitric oxide synthesis, which in turn mediates muscarinic cholinergic signaling in cardiac myocytes and specialized pacemaker tissue, and modifies contractile response to 13-adrenergic stimulation (page 217 bridging page 218) . One of ordinary skill in the art would have been expected to have a reasonable expectation of success in making and use such as the combined composition because the nicotinic acetylcholine receptor y subunit promoter of Gilmour et al. is proven to modulate gene expression as desired, upon electrical stimulation.

Thus, the Examiner concludes that the claimed invention was prima facie obvious at the time the invention was made.

In order to fulfill the requirements of an obviousness rejection the combined references must teach the invention as a whole. Further, the Examiner can not pick and choose elements of one reference and combine it with those with another reference when there are also clear teachings within the references that teach away from the claimed invention.

Gilmour et al. teaches that the nicotinic acetylcholine receptor *y* subunit promoter mediates **suppression** of transcription in response to electrical activity." (emphasis added). Applicants' current amendment clearly indicates that their invention is tied to enhancing transcription (the antithesis of suppression). The teaching of Gilmour for suppression of transcription can not be ignored. Even in light of Gilmour's teaching of suppression of transcription, the further addition of Kaye et al. in combination with Gilmour also fails to teach or describe the claimed invention.

Kaye's studies involve *in vitro* electrical stimulation of isolated heart myocytes. These cells were used in their native condition. No genetic engineering of the cells was performed or suggested (nor could Gilmour teach the use of

heterologous promoter constructs in engineered cells). Kaye's experiments suggest that the nitric oxide synthase gene would respond *in vitro* to pacing; however, the reference fails to adequately describe an implantable pacemaker tied to implanted cells that are genetically engineered to a heterologous system, and thus Kaye or Gilmour in view of Kaye fails to teach or suggest the claimed invention.

In view of the amendments to the claims, and the arguments submitted above distinguishing the present claimed invention over Gilmour and Kaye, Applicant respectfully request the present rejection be removed.

Conclusion

In view of the submitted amendments and discussion of the prior art, Applicants believe all the present rejections have been overcome, and respectfully request that the present application be allowed to issue.

Respectfully submitted,

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